

# TIME COURSE OF PHOTOSYNTHESIS IN APPLE LEAVES EXPOSED TO CONTINUOUS ILLUMINATION

R I C H A R D H . B Ö H N I N G

(WITH NINE FIGURES)

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## Introduction

In a number of previous investigations, the rate of photosynthesis has been measured in plants exposed to constant environmental conditions but the duration of the light period has always been relatively short, often fractions of natural day lengths. Most of the information relative to the effect of the duration of the light period upon photosynthesis has been gained from experiments conducted by investigators primarily interested in some other aspect of the process and in which they exposed plants to day lengths corresponding to the natural day or to light periods of only several hours duration. Interpretations of the data thus obtained have led to the general conception that even when all external environmental conditions are maintained constant and favorable to a high rate of photosynthesis, some internal factor might become limiting with time, resulting in a decline in rate.

It has been known for a long time that variations in temperature, light intensity, partial pressure of carbon dioxide in the atmosphere, and soil water supply have marked effects upon the rate of photosynthesis in land plants. Contrary to earlier beliefs it is now common knowledge that there is no specific optimal value for each of these factors. An "optimum" temperature at one light intensity, for example, may be suboptimal at another light intensity. All of these factors are so closely interrelated that the effects of one cannot be evaluated independently but due consideration must be given to all of the other factors in a final analysis of the data.

At either high temperatures or high light intensities inhibition in the rate of photosynthesis appears in a very short time. For that matter almost any factor, if present in excess, will become inhibitory. In previous investigations values for both temperature and light intensity have been found at which no inhibition in the rate of photosynthesis occurred within the periods of measurement, which varied in length from a few minutes to the natural daylight period. The fact that no decrease in the rate of photosynthesis occurred during such periods of investigation does not eliminate the possibility that a longer exposure to those same conditions might result in a gradual diminution in the rate of photosynthesis. Conversely more inhibition under a given set of conditions in a given period of time does not necessarily mean that inhibition will result under all conditions even if the time of exposure is lengthened.

No previous attempt has been made to ascertain experimentally how much longer the initial rate of photosynthesis could be maintained if a set of environmental conditions which did not result in inhibition within the natural daylight period, were maintained constant for a long period of time.

It was the purpose of this investigation to make "around the clock" measurements of the rate of photosynthesis over periods of some days' duration in apple leaves subjected to continuous illumination under controlled and favorable environmental conditions. The principal objective was to get an indication of the maximum length of time that photosynthesis could be maintained in an apple leaf if light were a continuous factor and all other factors were both adequate and constant. It was also hoped that this experimental procedure would yield information which would aid in an evaluation of the time-factor effect.

### Review of the literature

Fairly complete reviews of the literature on the subject of photosynthesis have been made by STILES (23), SPOEHR (22), MILLER (15), DUGGAR (7), and more recently by RABINOWITCH (19). SHIRLEY (21) has reviewed the investigations of the effect of light upon photosynthesis. In this paper only the literature which is directly related to this investigation will be discussed.

BLACKMAN and MATTHAEI (3) in a summation of the principles set forth by BLACKMAN (2) state that, "at moderate temperatures a leaf can maintain its assimilation at the maximal value continuously for a considerable time." They further state that, "the decline in the rate of photosynthesis from the initial value increases in magnitude with the higher temperatures." They conclude that, "these phenomena necessitate the introduction of a 'time factor' into assimilation values at high temperatures." They found that when a leaf was exposed to diffuse daylight only and if the temperature was low the rate of photosynthesis remained uniform in the varying light throughout the day until sunset.

As the above experiments were conducted under natural day lengths and not continuous illumination it is impossible to evaluate the expression "for a considerable time."

KOSTYCHEV, TSCHENOKOV and BAZYRINA (11) have shown that in certain species of plants in the Arctic region photosynthesis continues throughout the 24-hour day. The rate of photosynthesis in the Arctic plants investigated fluctuated through a daily cycle with the minimum occurring around midnight and the maximum around noon. These fluctuations could be correlated with similar fluctuations in temperature and light intensity.

MÜLLER (17) states that on the basis of data which he presents showing the effect of temperature and light intensity on the rate of apparent photosynthesis in *Chamaenerium latifolium* and in view of the fact that the light intensity at midnight in the first half of July in Godhavn, West

Greenland is about 450 lux it is certain that during this period photosynthesis occurs throughout the 24-hour day.

MITCHELL (16) made measurements of the rate of photosynthesis in attached leaves of several species of plants exposed to 12 hours of illumination at constant intensity daily and a temperature of 24.5–25.5° C. Hourly measurements were made over periods of three to eight hours for intervals varying from two to fourteen days. Data obtained from the experiments with tomato leaves show that the amounts of carbon dioxide utilized varied only slightly from hour to hour during a given period of illumination.

MEYER and ANDERSON (14) in a discussion of the effects of the duration of the light period upon photosynthesis state that “even if all external conditions remain favorable the amount of photosynthesis in a 12-hour light period will usually not be twice that in a six-hour period, because of certain internal factors which often become limiting after the process has been in progress for several hours.”

MEYERS and BURR (18) present families of curves showing changes in the rate of photosynthesis in *Chlorella* as a function of both intensity and duration of illumination. At 1000 foot candles they found the rate of photosynthesis to be constant and no inhibition occurred after 30 minutes of illumination. At 4000 foot candles inhibition occurred after 20 minutes causing a gradual reduction of photosynthesis to a final rate lower than that at 1000 foot candles. As the intensity was increased inhibition occurred sooner and was of greater magnitude. All of their data “are consistent with the view that with increasing intensities photosynthesis is progressively inhibited while photooxidation is progressively increased at a much lower rate.” The inhibition in rate of photosynthesis is considered to be “a destruction of some factor in the photosynthetic mechanism. The factor is reduced to a concentration at which it is maintained at a steady rate for any given light intensity.”

KRAMER and DECKER (12) state that “in preliminary experiments at 30° C the rate of photosynthesis in oaks began to decrease rapidly after five or six hours at a high light intensity.”

DECKER (5) found that in preliminary measurements of the rate of photosynthesis in red and loblolly pines at temperatures of 20°, 30°, and 40° C the rate remained unchanged for at least six hours at a light intensity of 4500 foot candles.

RABINOWITCH (19) points out that while the ascending parts of the light curves of shade plants and sun plants are “independent of the duration of illumination the parts of these curves corresponding to saturating light intensities, often are time-dependent.” “In ‘shade plants’, a decline in the rate of photosynthesis at high light intensities cannot be avoided even in rapid experiments; if the illumination is extended, a complete inhibition may ensue and the plants may suffer an irreversible light injury, or even death by ‘sunstroke’. In typical ‘sun plants’, on the other hand, a rapid determination may lead to light curves with a com-

pletely flat 'saturation plateau' extending far beyond saturating light intensity; however a sufficiently prolonged and intense illumination is bound to produce a gradual inhibition in these plants as well."

### Materials and methods

The method used in this investigation to measure the rate of apparent photosynthesis is a modification of that employed by HEINICKE and HOFFMAN (10). The apparatus is diagrammed in figure 1. It consists of a temperature control cabinet (H), two leaf cups (D), a tank of compressed air (I), two sets of control valves for regulating air flow (J), three Sargent Wet Test Meters (F), four absorption towers (K), four reservoir flasks (L), a vacuum pump, and an overhead source of distilled water for washing down the towers. Where a unit is duplicated in the apparatus only one is represented in the diagram. Several features used by DECKER (5) were employed in the construction of the apparatus used in this investigation.

The cabinet used to maintain constant environment was approximately  $4 \times 4 \times 4$  feet. The sidewalls of the cabinet were insulated with a three-inch layer of rock wool. The bottom and top, except for the area occupied by the lights, were also insulated with a three-inch layer of glass wool.

Temperature control was maintained by means of a cooling unit in the cabinet connected to a General Electric refrigerant condensing unit mounted beneath the cabinet, and a high resistance wire heating unit connected to thermo-regulators. Record of the temperature was kept by a hygrothermograph and a minimum-maximum thermometer inside the cabinet. Temperature was maintained at  $25^{\circ} \text{C} \pm 1.5^{\circ} \text{C}$ . Leaf temperature was measured by means of a copper-constantan thermocouple and was found to be within  $0.5^{\circ} \text{C}$  of the prevailing temperature in the cabinet.

Illumination was provided by nine General Electric Mazda 300 Watt reflector flood lamps (C), suspended from a tubular framework mounted on top of the cabinet (G). Each light socket was equipped with a ball joint (B) and fastened to the framework by means of a U-strap with wing nuts on each side (A). This made it possible to move the lamps in a horizontal direction along the pipes, or focus them through a circle of  $360^{\circ}$ . By varying the number and wattage of the lamps, or by using spot lamps instead of flood lamps, any desired light intensity up to 10,000 foot candles could be obtained. The amount of infrared radiation which entered the cabinet was reduced by filtering the light through a  $2\frac{1}{4}$  inch layer of cold water flowing through a water bath (E). A continuous record of light intensity was kept by means of a pyreheliometer placed in the cabinet and connected to a Micromax recorder. The intensity of light falling on the leaves attached to the leaf cups was measured by a Weston Illumination Meter.

General ventilation was provided by air flowing into the cabinet from the compressed air line at the rate of 100 liters per minute. This rate of flow caused a replacement of air in the cabinet approximately every 20

minutes and was sufficient to supply at least 2.5 liters of fresh air per square centimeter of leaf surface per hour. Because of the usual fluctuations in  $\text{CO}_2$  content of the atmosphere, it was desirable in later experiments to work with a source of air in which the  $\text{CO}_2$  content of the air passing through the leaf cups could be maintained constant. An air stream with nearly constant  $\text{CO}_2$  content was provided by means of a tank of compressed air. The  $\text{CO}_2$  contents of two tanks of compressed air are rarely the same, but during the period in which the air from one tank was being used, a nearly constant value for the  $\text{CO}_2$  content was obtained. A tank of compressed air at 2,000 pounds pressure contains approximately 200 cubic feet of air and this lasts, with the rate of flow used in these experiments,

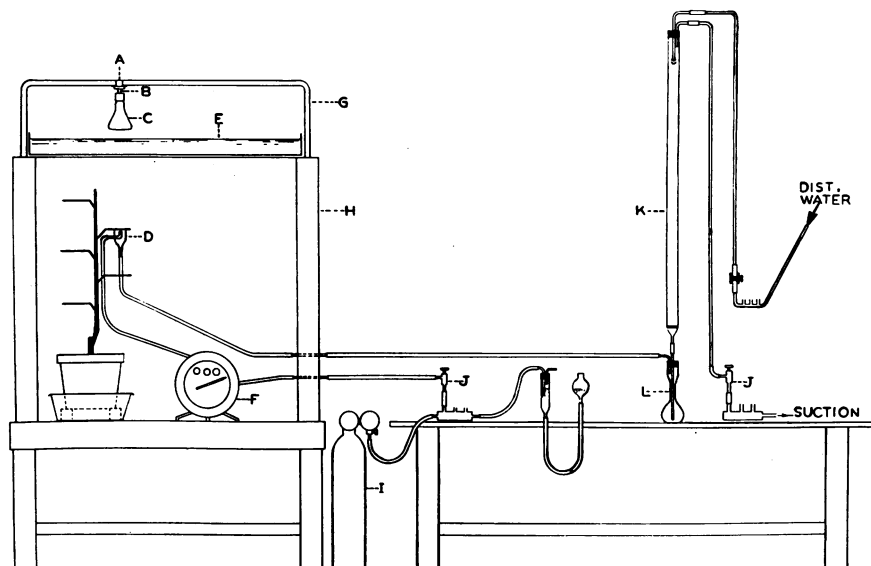


FIG. 1. Diagram of the apparatus used to measure the rate of apparent photosynthesis and to maintain the controlled environment. The lettered parts are described in the text.

for about 40 hours. At the end of 40 hours the empty tank was replaced by a full tank and the measurements continued. This procedure was followed for as long as it was desired to make continuous measurements.

It was found by SCHNEIDER and CHILDERS (20) that changes in water content of the soil have a marked effect upon the rate of photosynthesis. It is therefore necessary in any studies of this type to maintain soil moisture as constant as possible. The soil in the pots was kept continually moist by means of a constant watering device. The pot containing the experimental plant was placed in a large pan equipped with an inlet tube connected to a water supply, and the water was maintained at a height of one inch above the base of the pot. No attempt was made to control the relative humidity, as it has been shown by MCALISTER (13) and MITCHELL (16) that ordinary fluctuations in relative humidity have no apparent effect upon the rate of

photosynthesis in certain species of plants. Therefore, it was felt that fluctuations in relative humidity would have no appreciable effect upon the rate of photosynthesis in full-grown apple leaves since during the course of these experiments the relative humidity within the cabinet remained within the range of 40–60 per cent.

The procedure used in making the actual measurements of photosynthesis was as follows. One apple tree was placed in the temperature control cabinet at 7 p.m. of the evening previous to the date when the measurements were to begin. At 7 a.m. the following morning the leaf cup

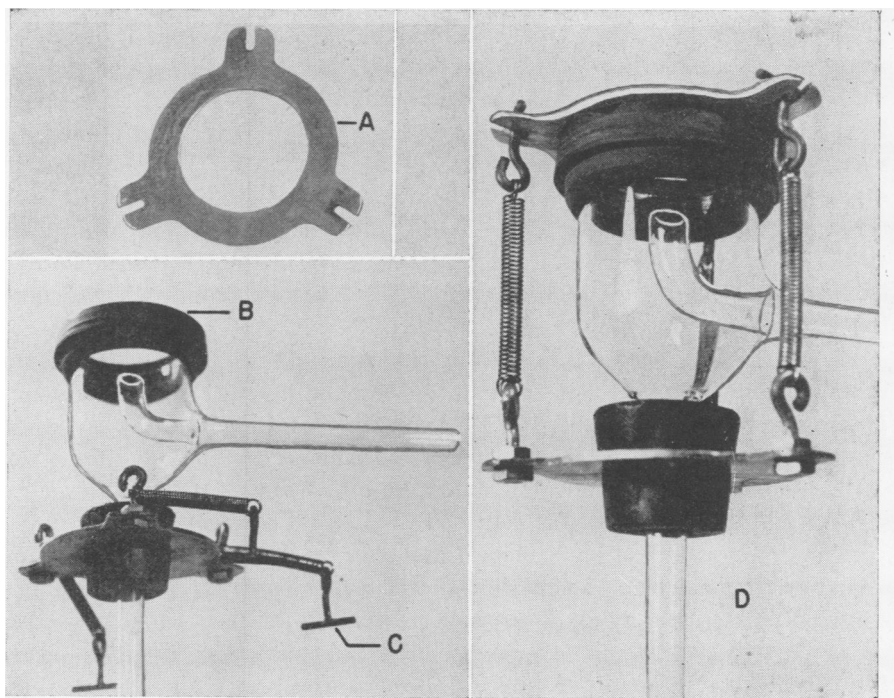


FIG. 2. The leaf cup arrangement as used in this investigation. The leaf cup is attached to the leaf in the following manner. A layer of grafting wax is placed on the rubber gasket (B). The leaf is then laid in position and pressed firmly against the grafting wax making an air-tight seal. The three-arm aluminum ring (A) is then laid over the leaf and the T-clips (C) of each spring placed in the slot in each arm. Final assembly of the leaf cup is shown in (D). The entire assemblage was supported in position by means of a support stand and suitable clamps so that the leaf was in a horizontal position.

was attached to the leaf. This was done by first placing a layer of grafting wax on the rubber gasket and then pressing the leaf firmly against the wax. The leaf cup was held in place by means of a clamp attached to a support stand as the leaf could not bear the weight of the cup. The leaf was held against the grafting wax seal by a three-arm aluminum ring, attached by springs to a similar ring below the leaf cup. This method of holding

the leaf in place obviated the danger of compressing the veins, as the tension on the leaf could be regulated by changes in the position of the ring below the leaf cup. As an added precaution against undue compression a slot had been previously cut in the rubber gasket to receive the midvein. The leaf cup arrangement is shown in figure 2.

The absorbent flasks containing 200 ml. of approximately 0.1N NaOH were put in place and all connections checked to insure no leaks. At 8 a.m. the lights in the cabinet were turned on and measurements begun. The air from the tank of compressed air flowed into a manifold with four outlets, three of which were equipped with needle valves to control the rate of flow to each of the two leaf cups and the check. The fourth outlet was connected to a glass tube which dipped 1 cm. below the surface of water in a reservoir. This simple device acted as a safety valve so that the pressure in the line never exceeded 1 cm. of water. The air entering the manifold was thus broken up into three streams; one serving as the check and the other two flowing through each of the two leaf cups. The air was metered through a Sargent Wet Test Meter in each line. The meters were read at the end of each run to ascertain the actual amount of air which passed over the leaves and through the check meter. The air passed from each gas meter to an absorption tower in each line.

THOMAS (24) shows that within the range of air flow used in this investigation the absorbent in an absorber similar to that described by Heinicke and Hoffman is 97–100 per cent. efficient. Preliminary tests were made to measure the efficiency of the absorbent under the conditions prevailing in this experiment. It was found by connecting two towers in series that all of the  $\text{CO}_2$  was removed in the first tower.

The towers were Pyrex glass tubes 35 mm. OD and 150 cm. long, sealed at the base to a Buchner funnel of the same diameter containing a sintered glass disc having uniform pores of 40 microns which served to break the air stream up into very small bubbles. The air then passed from each absorption tower into another manifold with three inlets, each equipped with a needle valve. The manifold was connected through the single outlet to a vacuum pump which was used to raise the absorbent solution in the towers. The rate of air flow from the tank was adjusted to slightly exceed the rate of exhaustion of air through the absorption towers by the vacuum pump. To prevent any excessive building up of pressure which would break the seal around the leaves the safety valve, previously mentioned, was installed in the line.

At the end of each run the vacuum pump and the air pressure were turned off and the solution allowed to drain from the towers back into the absorbent flasks. These flasks were 500 ml. volumetric flasks with an expanded neck of the type used in beet sugar industry and eliminated the need of transferring the solution from an absorbent flask to a volumetric flask at the end of the measurements. The meters were read, after which the towers were washed down with distilled water from the overhead

source. The distilled water entered a manifold on the side of the rack containing the bank of absorption towers. Each outlet of this manifold was connected by means of a short piece of Tygon tubing to a glass tube which ran to the top of the rack and connected by means of a short piece of rubber tubing to a glass tube protruding at the top of each absorption tower through a two-hole rubber stopper. The tube which entered the absorption tower had a bulb at the lower end with small holes around the circumference which served to spray the water in small streams over the towers, washing their walls as it flowed down the sides. A similar method of washing the towers is described in more detail by CHILDERS, *et al.* (4). After washing down the towers the flasks were removed and 10 ml. of saturated  $\text{BaCl}_2$  solution added to each flask. The flasks were then filled to mark with distilled water from the same source as used in washing down the towers, tightly stoppered, and shaken. After the precipitate had settled, a 100 ml. aliquot of the supernatant liquid was titrated with standard hydrochloric acid of approximately 0.1N, using phenolphthalein as the indicator. Calculation of the amount of  $\text{CO}_2$  absorbed was then made according to the method outlined by HEINICKE and HOFFMAN (10).

Two year old Yellow Transparent apple trees growing in 12-inch clay pots were used in these experiments. At the time of planting, the nursery stock was cut back to 10 inches above the surface of the soil, and the root systems were pruned. Three vigorous shoots were allowed to develop from the stub of the scion. The trees grew in the greenhouse from the date of transplanting, April 16, 1947, until they were placed out-of-doors on May 5, 1947. When it was time to make the measurements a vigorous tree was selected and placed in the cabinet as outlined under Methods. All measurements were made on fully expanded, attached leaves.

## Results and discussion

### EXPERIMENT 1

In experiment 1 a leaf of the Yellow Transparent apple tree was exposed to continuous illumination of approximately 5800 foot candles from 8 o'clock in the morning of June 26, 1947 until noon of July 13, 1947. The light intensity which was 6000 foot candles at the start of the experiment had decreased to 5600 by the end of the experiment. Continuous measurements of the rate of apparent photosynthesis in the fully expanded attached leaf were made during successive four-hour periods from the start of the experiment until 4 p.m. on July 2. During the period from 4 p.m. July 2 until 8 a.m. July 9 no measurements were made. During this period the plant remained exposed to the continuous illumination, air flow remained constant, and distilled water was substituted for the sodium hydroxide in the absorption towers. At 8 a.m. on July 9 measurements were resumed for successive four-hour periods until the termination of the experiment at noon July 13. The rate of photosynthesis was also measured in a dupli-



cate leaf but the Wet Test meter was subsequently found to be defective, and these data were discarded. The results of experiment 1 are presented in figure 3. The rate of apparent photosynthesis is expressed in terms of the number of milligrams of carbon dioxide utilized per square decimeter of leaf surface per hour. The rates of photosynthesis in apple leaves obtained in this and subsequent experiments are of the same order of magnitude as those reported by other investigators (HEINICKE and HOFFMAN, 10, and SCHNEIDER and CHILDERS, 20). The carbon dioxide concentration of the air before passing over the leaf expressed as milligrams per liter is also shown for each corresponding period for which the rate of apparent photosynthesis was measured. All volumes of air used in the calculations were corrected to 20° C and 760 mm Hg.

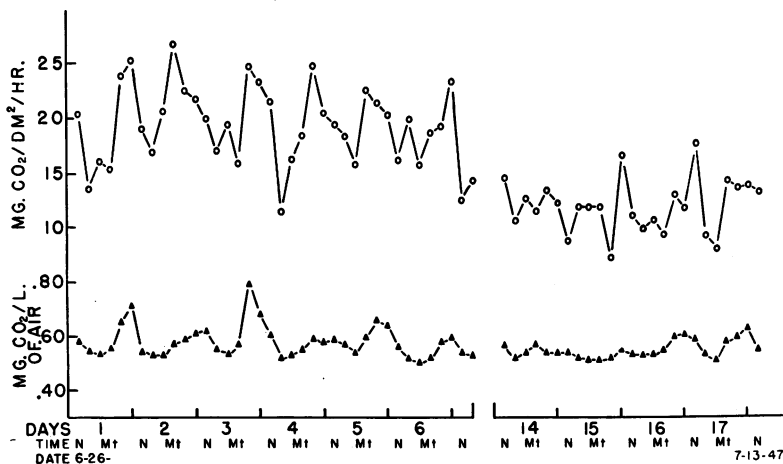


FIG. 3. Rate of apparent photosynthesis in an individual leaf of apple exposed to continuous illumination of 5800 foot candles.

The rate of apparent photosynthesis was found to fluctuate through a daily cycle with the maximum rate occurring during the early morning hours and the minimum rate occurring during the mid-afternoon hours. The fluctuation in rate of apparent photosynthesis can be correlated with a similar fluctuation in the carbon dioxide concentration of the atmosphere. This correlation demonstrates the limiting effect of the carbon dioxide concentration of the atmosphere upon the rate of photosynthesis under the conditions of this investigation. Regardless of the amplitude of the fluctuations it is apparent from the data that during the first six days of exposure to continuous illumination there is no downward trend. During the period of 14 to 17 days exposure to continuous illumination a definite decline in the mean rate of apparent photosynthesis occurred while the mean carbon dioxide concentration remained approximately the same as during the first six days.

The rates of apparent photosynthesis calculated from each of the six successive four-hour measurements during each 24-hour period of exposure to continuous illumination are averaged to obtain an average hourly rate

of photosynthesis per day. A similar average is made of the values for the carbon dioxide concentration of the air (fig. 4).

The average hourly rate of apparent photosynthesis per day remained fairly uniform during the first six days. The variation from the average was 8.1 per cent. and the maximum variation was only 12.6 per cent. Small fluctuations which did occur can mostly be attributed to similar fluctuations in the carbon dioxide concentration of the air. During the 14th to 17th days of exposure to continuous illumination the rate of apparent photosynthesis again remained fairly uniform but the mean value was 38.6 per cent. lower than during the first six days of exposure whereas the mean carbon dioxide concentration of the atmosphere had decreased only five per cent.

When the leaves were examined at the end of 18 days exposure to

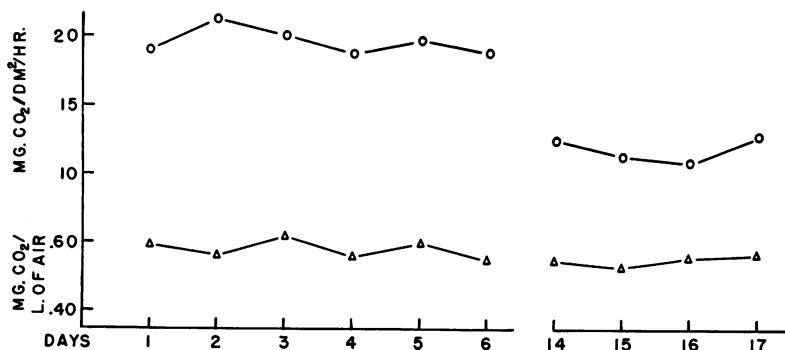


FIG. 4. The average hourly rate of apparent photosynthesis per day in an individual apple leaf exposed to continuous illumination of 5800 foot candles. Each point is the average of six 4-hour measurements.

continuous illumination of 5800 foot candles it was found that marked bleaching of the chlorophyll had occurred. The portion of the leaf which was shaded by the three-arm aluminum ring was still dark green. Some necrotic areas were present where the leaf was in contact with the grafting wax. The decline in rate of apparent photosynthesis can probably be attributed to the reduced chlorophyll content of the leaf.

## EXPERIMENT 2

Because of the bleaching of the chlorophyll occurring in the leaves exposed to continuous illumination of 5800 foot candles a lower light intensity of 3200 foot candles was used in experiment 2. This is well above the saturation light intensity for apple, which according to HEINICKE and HOFFMAN (10) is 1500 foot candles and according to ALLMENDINGER, *et al.* (1) is 1200–1400 foot candles under favorable outdoor conditions. The results of experiment 2 are presented in figure 5.

The lower values for apparent photosynthesis obtained in this experiment, as compared with the preceding experiment, cannot be attributed to

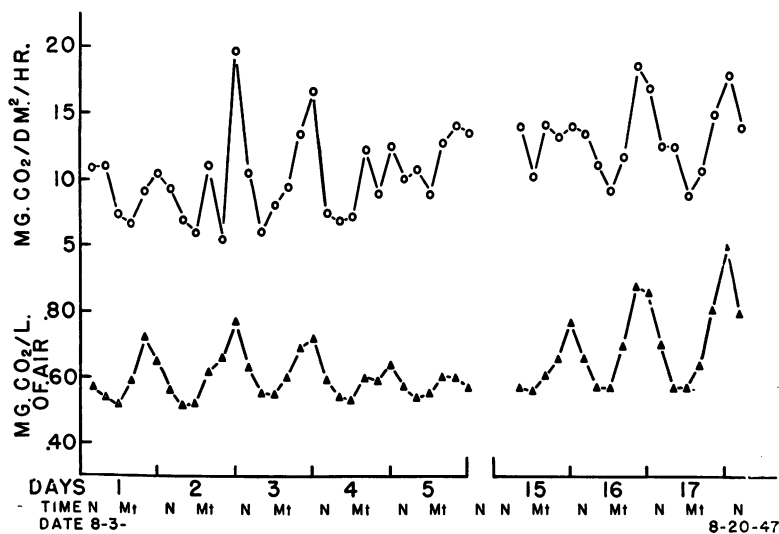


FIG. 5. Rate of apparent photosynthesis in leaves of apple exposed to continuous illumination of 3200 foot candles. Each point is the average of two leaves.

the limiting effect of light but are probably due to usual differences in rate occurring in different leaves on the same tree or may be due to a decrease in the efficiency of the photosynthetic mechanism accompanying an increase in age.

The procedure used in making the measurements was the same as in experiment 1. The plants were exposed to continuous illumination from 8 a.m. August 3, 1947 until noon August 20, 1947. Actual measurements were made during the periods from 8 a.m. August 3 until 8 a.m. August 8, and from noon August 17 until noon August 20.

As in experiment 1 a daily cycle in the rate of apparent photosynthesis

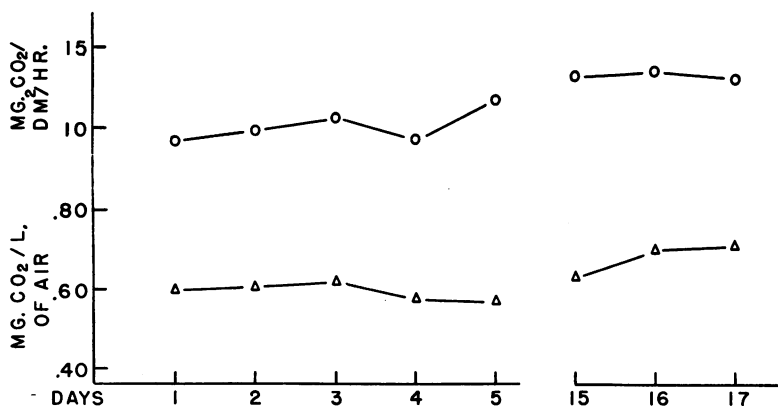


FIG. 6. The average hourly rate of apparent photosynthesis per day in leaves of apple exposed to continuous illumination of 3200 foot candles. Each point is the average of twelve four-hour measurements.

occurred which can be correlated with a similar periodicity in the carbon dioxide concentration of the atmosphere. There was no downward trend, however, as occurred during the 18 days exposure to 5800 foot candles. Data showing the average hourly rate of apparent photosynthesis per day in the apple leaves exposed to 3200 foot candles are presented in figure 6.

As in experiment 1 it is apparent that the average hourly rate per day remained fairly uniform. There was an increase of 30.4 per cent. in the mean rate of photosynthesis in the 14-17 day period over that of the mean rate in the 1-5 day period. This can be correlated with an increase of 14.2 per cent. in the mean carbon dioxide concentration of the atmosphere. Data of DECKER (6) indicate that in certain species of pine and hardwoods an increase of one per cent. in the carbon dioxide concentration of the atmosphere will bring about an increase of approximately three per cent. in the rate of apparent photosynthesis.

In this investigation it was found that in apple leaves exposed to continuous illumination an increase of one per cent. in the carbon dioxide concentration of the atmosphere was followed by an increase of approximately two per cent. in the rate of apparent photosynthesis during the first few days of exposure. As the length of exposure was increased the relationship was in the order of 1-1. In later experiments in which apple trees that had developed in the greenhouse under conditions of low light intensity were used, a negative relationship was found, that is, the rate of apparent photosynthesis was decreasing at a time when a marked increase in the concentration of carbon dioxide was occurring.

Only slight bleaching of the chlorophyll occurred in the leaves exposed to continuous illumination of 3200 foot candles.

### EXPERIMENT 3

Because of the daily fluctuations in the concentration of carbon dioxide in the atmosphere, experiment 3 and all subsequent experiments were conducted using tanks of compressed air to provide an air stream having a constant carbon dioxide concentration. Light intensity in this experiment was 5200 foot candles. The measurements were made continuously for each successive four-hour period from noon on September 3, 1947 until noon on September 11, 1947. The results of experiment 3 are presented in figure 7.

When all external environmental conditions are maintained constant it is apparent from the data that marked fluctuations in the rate of apparent photosynthesis do not occur. The rate of apparent photosynthesis remained fairly uniform during the three days exposure to continuous illumination. On the second day a new tank of air having a slightly higher carbon dioxide concentration than the first was placed in the line and the mean rate of apparent photosynthesis was found to show a corresponding increase.

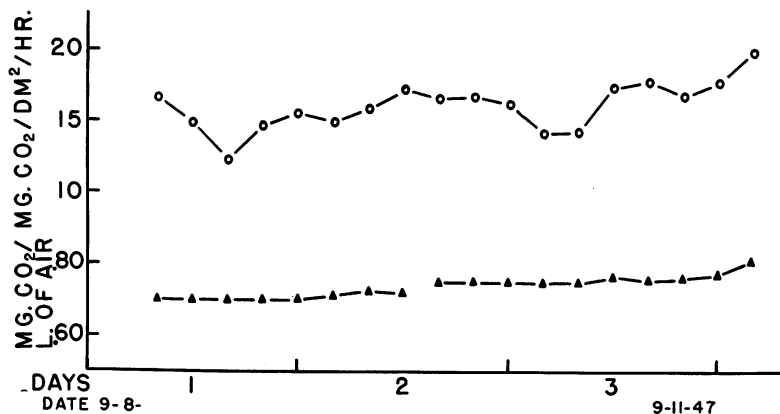
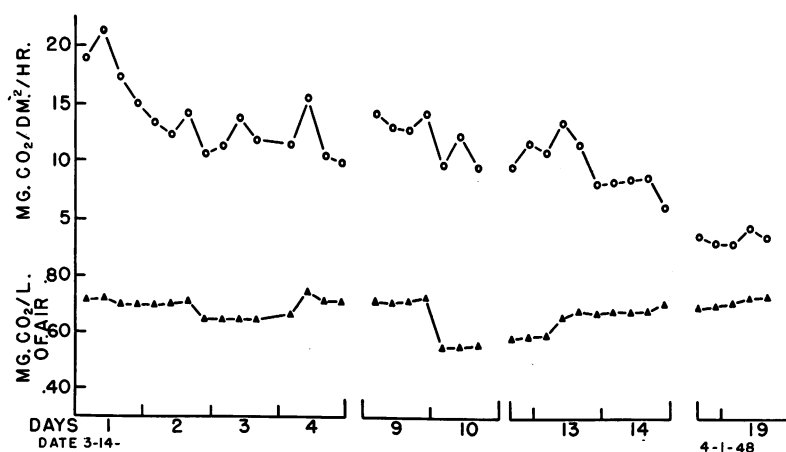


FIG. 7. Rate of apparent photosynthesis in apple leaves exposed to continuous illumination of 5200 foot candles.

#### EXPERIMENT 4

The apple trees used in the first three experiments were left out-of-doors at all times except when they were actually being used in experiments. On January 17, 1948 five of the trees were brought into the greenhouse, repotted, cut back, and allowed to develop as described previously. The leaves developed under the conditions of low light intensity prevailing in the greenhouse during the winter months and presumably had the physiological characteristics of shade leaves. Hereinafter the leaves used in all previous experiments will be referred to as "sun" leaves and those used in this experiment will be referred to as "shade" leaves. The rate of apparent photosynthesis was measured in the "shade" leaves exposed to continuous illumination of 3800 foot candles. The results of experiment 4 are presented in figure 8.



It is apparent from the data that in the "shade" leaves exposed to continuous illumination of only 3800 foot candles there is a marked initial falling off in the rate of apparent photosynthesis which did not occur in the "sun" leaves at 5800 foot candles. The average hourly rate of apparent photosynthesis per day for those days on which the carbon dioxide concentration of the compressed air was very nearly the same was calculated from the data in figure 8 and are presented in figure 9.

The rate of photosynthesis decreased to a minimum value by the second day and then levelled off, remaining fairly uniform until the tenth day. On the tenth day the rate again began a gradual falling off and had fallen to zero in one leaf by the eighteenth day. At the end of the experiment the leaves were examined and it was found that marked bleaching had occurred.

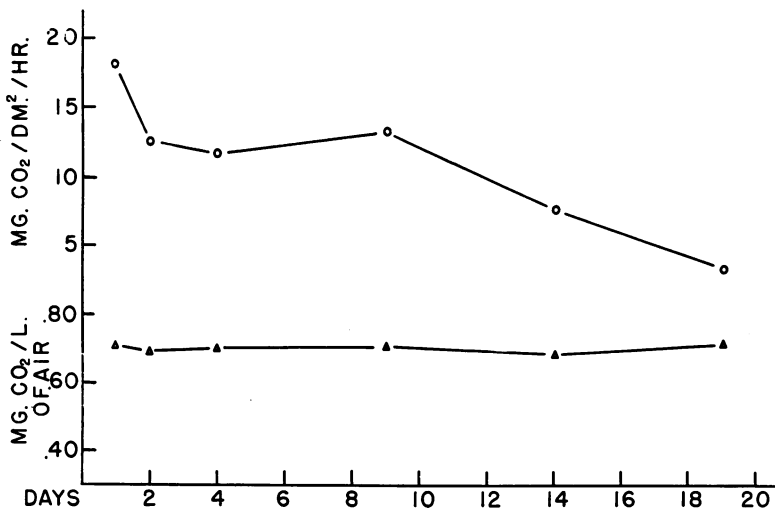


FIG. 9. The average hourly rate of apparent photosynthesis per day in "shade" leaves of apple exposed to continuous illumination of 3800 foot candles. Each point is the average of eight six-hour measurements.

Necrotic areas were also beginning to appear in the bleached regions. Pronounced necrosis was also evident where the leaves were in contact with the grafting wax.

In this investigation all external environmental factors which have been shown to have a pronounced effect upon the rate of photosynthesis were maintained constant at values conducive to a usual rate of photosynthesis in apple leaves. The possibility of variations in any of the external factors which would cause fluctuations in the rate was thereby removed. When a constant rate of photosynthesis obtains under constant environmental conditions no explanation seems necessary. It is only when the rate of photosynthesis declines, rises, or periodically fluctuates when environmental conditions are constant, that an interpretation of the data may lead to a possible explanation of the cause or causes of such phenomena.

The constant environmental conditions maintained in this investigation preclude any external factor from varying in such a way as to cause a decline in the rate of photosynthesis. These same conditions however may directly or indirectly affect some internal factor which could exert a limiting effect upon the rate of photosynthesis.

A number of internal factors may account for the decrease in rate of photosynthesis observed in the "sun" leaves by the fourteenth day of continuous exposure to a light intensity of 5800 foot candles. One possibility is that an accumulation of the end products of the photosynthetic reaction may have caused the falling off. Satisfactory evidence on this point however is not available. As is apparent from the review of the literature on the subject presented by RABINOWITCH (19), some investigators report that the accumulation of the end products of photosynthesis may exert an inhibitory effect, others, no effect, and still others, an accelerating effect.

The possibility that an accumulation of the end products might result in an increase in the rate of respiration thereby resulting in a decrease in the rate of apparent photosynthesis cannot be ignored. However, in this investigation the leaves remained attached to the stem and the products of photosynthesis were probably for the most part translocated to the rapidly growing stem tip and consumed in respiration and assimilation. The accumulation of end products is, therefore, not considered to have occurred because translocation of the end products of photosynthesis from the leaves had not been restricted.

MEYER AND ANDERSON (14) point out that a decrease in photosynthesis may be brought about by a reduction in the water content of leaves because it may cause either a "decrease in the diffusive capacity of the stomates" or a "reduction in the hydration of the chloroplasts and other parts of the protoplasm."

In these experiments the plants were watered by means of a constant watering device to minimize the possibility of an internal water deficit. Wilting was not observed in any of the leaves on the plants so it is very unlikely that the water content of the leaves ever became reduced enough to have any appreciable effect upon the rate of photosynthesis.

RABINOWITCH (19) suggests that the inactivation of photosynthesis by intense light can be attributed to a photooxidation of one of the enzymes in the photosynthetic mechanism. In strong light the destruction of the enzyme may result in a complete cessation of photosynthesis. In moderate light the inhibition may remain incomplete because the enzyme is continuously restored by the organism, so that after some time, an equilibrium may be reached between the rate of its destruction by photooxidation and of its restoration by metabolic processes. The rate of photosynthesis may then remain uniform at a rate corresponding to the stationary concentration of the enzyme.

MYERS AND BURR (18) consider that the first few minutes of illumination in which the rate of photosynthesis decreases to a constant final value,

represents the photooxidation of one of the "photosynthetic enzymes" to a concentration at which it is maintained at a steady rate for any given light intensity. A further discussion of this paper may be found in the section containing a review of the literature.

As the "sun" leaves had developed out-of-doors under conditions of high light intensity it is possible that an equilibrium had been reached between the destruction and formation of the "photosynthetic enzyme" for the light intensity to which they were exposed during the experimentation. This would account for the failure of a decline in rate of photosynthesis to appear in the "sun" leaves during the first few days of exposure to continuous illumination.

The diminution in rate of photosynthesis in the "sun" leaves is probably more closely associated with the decreased chlorophyll content of the leaves, resulting from bleaching of the chlorophyll, than with any other single factor.

An explanation of the initial decrease in rate of apparent photosynthesis observed in the "shade" leaves followed by a secondary decline beginning on the tenth day of exposure to continuous illumination of 3800 foot candles involves a consideration of the same internal factors discussed in connection with the "sun" leaves.

The accumulation of the end products is not considered to have occurred for two reasons. First, translocation had not been restricted and second, it does not seem plausible that in the "shade" leaves there would be an initial accumulation followed by a second accumulation 10 days later.

Wilting was not observed in any of the "shade" leaves so the effect of an internal water deficit does not seem to warrant further consideration, having been discussed previously in connection with the "sun" leaves.

The light intensity to which the "shade" leaves were exposed was only 3800 foot candles and would not be considered intense light. It is possible, however, that prolonged continuous exposure of "shade" leaves to such a light intensity, which was somewhat higher than that under which they developed, will have the same effect as a shorter exposure to intense light. Photooxidation of one of the "photosynthetic enzymes" therefore might well be the underlying cause for the initial reduction in the rate of photosynthesis in the "shade" leaves.

The secondary decline in rate of photosynthesis in the "shade" leaves following the tenth day of exposure to continuous illumination of 3800 foot candles is probably the result of the decreased chlorophyll content of the leaves, resulting from bleaching of the leaves and the appearance of necrotic areas.

The differences in results obtained with "shade" leaves and "sun" leaves indicate that the previous history of the leaves and the light intensity used in the investigation are both important in determining the length of time that the initial rate of photosynthesis can be maintained. Similar results were obtained by HARDER (8) who found that the shape of the



photosynthetic curve for *Fontinalis* sp. was dependent upon the ratio of the light intensity under which the plants had grown previous to the investigation to the light intensity to which they were exposed during the investigation.

### Summary

Continuous measurements were made of the rate of apparent photosynthesis in fully expanded attached leaves of apple exposed to continuous illumination over periods of three to 18 days.

The intensity of the continuous illumination was maintained constant for any one experiment. In some of the experiments the leaves were exposed to the usual atmospheric fluctuations in carbon dioxide and in others the carbon dioxide concentration of the air actually passing through the leaf cups was maintained constant. Temperature was maintained at  $26^{\circ}\text{C} \pm 1.5^{\circ}$ .

When the leaves were exposed to the usual atmospheric fluctuations in carbon dioxide a daily cycle in the rate of apparent photosynthesis was found to occur, which could be correlated with a similar fluctuation in the carbon dioxide concentration of the atmosphere. The cyclic variation in rate of apparent photosynthesis did not occur when tanks of compressed air, in which the carbon dioxide concentration was nearly constant, were used.

In "sun" leaves of apple the rate of apparent photosynthesis was fairly uniform for at least 18 days under continuous illumination of 3200 foot candles. At a light intensity of 5800 foot candles the rate had decreased about 40 per cent. by the fourteenth day.

In "shade" leaves of apple exposed to continuous illumination of 3800 foot candles a sharp initial decline in the rate of apparent photosynthesis occurred, reaching a minimum value by the second day. This value was maintained until the tenth day. Following the tenth day a further steady falling off in rate set in approaching almost a zero value by the twentieth day.

Bleaching of the chlorophyll was apparent at all light intensities but was most pronounced at the high intensities.

A possible explanation of the cause of the decrease in rate of photosynthesis observed in some of the experiments is presented. A number of internal factors which might account for the declines in rate are discussed. It is suggested that the initial falling off in rate in the "shade" leaves is probably the result of the destruction of one of the "photosynthetic enzymes." The final decline in photosynthetic rate in the "shade" leaves and the diminution in rate occurring in the "sun" leaves is attributed to the reduced chlorophyll content of the leaves resulting from bleaching of the chlorophyll.

Several basic generalizations can be drawn from this investigation. The length of time that the initial rate of photosynthesis can be maintained in apple leaves exposed to continuous illumination of such intensity that

light is not a limiting factor, will be influenced by the intensity of the illumination and the previous history of the leaves. In apple leaves which have developed under conditions of high light intensity the initial rate of photosynthesis can be maintained for at least 18 days under continuous illumination of 3200 foot candles at a temperature of 25° C. In apple leaves which have developed under conditions of low light intensity there is a rapid initial decline in rate under continuous illumination of 3800 foot candles to a constant value which can be maintained for a relatively long period. Prolonged exposure of "shade" leaves to continuous illumination of even relatively low light intensities will result in bleaching of the chlorophyll and finally in cessation in the rate of photosynthesis.

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